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Review

Chromosome set manipulation and sex control in common carp: a review

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Abstract

The development of techniques for production of gynogenetic, androgenetic, polyploid, and monosex progenies in common carp (*Cyprinus carpio* L.) is described from a chronological perspective. Gynogenetic progenies were obtained either by suppression of the second meiotic division in eggs (meiotic gynogenesis) or by suppression of the first mitotic division in haploid embryos (mitotic gynogenesis). As a rule, gynogenetic progenies of common carp were all-female, revealing female homogamety (females—XX, males—XY) in this species. Induced gynogenesis results in increased homozygosity; the rate of increase depends on the type of gynogenesis. Inbreeding coefficient (F) for one generation of meiotic gynogenesis in common carp is about 0.6, while diploids obtained by mitotic gynogenesis are homozygous for all genes ($F = 1.0$). Mitotic gynogenesis was used for production of clones in common carp. In androgenetic progenies of common carp, YY males were identified, that after crossing with normal females (XX) produced all-male progenies. Triploids of common carp are characterized by a significant reduction in gonad development (especially ovaries). However, the reduction in gonad development did not result in an increase of somatic growth rate of fish. The procedure for androgen treatment to induce phenotypic sex reversal in genotypic females (XX) was elaborated. All-female progenies of common carp were produced on a large scale by crossing normal females (XX) with hormonally sex-reversed males (XX). Rearing of all-female progenies in conditions when fish normally reach sexual maturity before reaching of market size increased production yield by 7–8%. In a few cases distant hybridization resulted in polyploidy of fish without application of any physical treatment. The ability of hybrid females between crucian carp (*Carassius auratus*) and common carp to produce diploid (with unreduced chromosome number) gametes resulted in opportunities to produce triploid and tetraploid hybrid progenies.

Résumé

Manipulation chromosomique et contrôle du sexe chez la carpe commune : une synthèse. Le développement de techniques de production de lignées gynogénétiques, androgénétiques, polyploïdes et monosexes est décrit pour la carpe commune (*Cyprinus carpio* L.) sous l'aspect chronologique. Des lignées gynogénétiques ont été obtenues soit par suppression de la 2^{e} division méiotique des œufs (gynogenèse méiotique) ou par suppression de la 1re division mitotique des embryons haploïdes (gynogenèse mitotique). En règle générale, les descendants gynogénétiques de la carpe commune sont toutes femelles, révélant une homogamétie femelle (femelles—XX, mâles—XY) chez cette espèce. Une gynogenèse induite provoque une augmentation d'homozygotes ; ce taux d'accroissement dépend du type de gynogenèse. Le coefficient de consanguinité (F) est environ 0,6 pour une génération de gynogenèse méiotique chez la carpe commune, tandis que les diploïdes obtenus par gynogenèse mitotique sont homozygotes pour tous les gènes ($F = 1,0$). La gynogenèse mitotique a été utilisée pour la production de clones chez la carpe. Chez les descendants androgénétiques, les mâles YY ont été identifiés, après croisement avec des femelles normales (XX) et produisent uniquement des mâles. Les triploïdes sont caractérisés par une réduction significative du développement des gonades (des ovaires en particulier). Cependant, la réduction du développement des gonades ne provoque pas une augmentation du taux de croissance somatique du poisson. La procédure du traitement androgène pour induire l'inversion sexuelle en femelles (XX) a été élaborée. Les descendants toutes femelles ont été produites à grande échelle en croisant des femelles normales (XX) avec des mâles inversés sexuellement au moyen d'hormones (XX). L'élevage de descendants toutes femelles, lorsque les poissons atteignent normalement leur maturité sexuelle avant d'atteindre leur taille commerciale accroît le rendement de production de 7–8 %. Dans quelques cas, des hybridations entraînent une polyploïdie sans application de quelque traitement physique. La capacité des femelles hybrides entre *Carassius auratus* et la carpe commune

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pour produire des gamètes diploïdes (avec un nombre non réduit de chromosomes) a donné l'occasion de produire des descendants triploïdes et tétraploïdes hybrides.

Keywords: Gynogenesis; Androgenesis; Polyploidy; Sex control; Common carp

1. Introduction

The goal of this article is to provide a review of information on chromosome set manipulation methods and sex regulation in common carp. Chromosome set manipulation methods include induced gynogenesis, androgenesis, and polyploidy. Artificial sex regulation in common carp, as well as in other fish species, is connected mainly with elaboration and application of the hormonal sex reversal method. Common carp is a traditional subject for chromosome set manipulation studies. It is a popular aquaculture species with established methods of artificial spawning and rearing. Common carp is a convenient subject for these types of investigations since it has high fecundity and unique morphological genetic markers such as genes for scale cover types and color mutations. The development of techniques for production of gynogenetic, androgenetic, polyploid, and monosex progenies in common carp is described in this review from a chronological perspective.

Before presenting information on common carp, a brief general description of each method is provided. More detailed general information may be found in reviews on chromosome set manipulation and sex control in fish (Cherfas, 1981; Thorgaard, 1983; Chourrout, 1987; Ihssen et al., 1990; Hunter and Donaldson, 1983; Purdom, 1993).

2. Induced gynogenesis

Gynogenesis is embryo development under control of only maternal heredity after activation of eggs by insemination. Natural gynogenesis has been described in several all-female forms of fish (Cherfas, 1981; Purdom, 1993). Gynogenetic progenies may be obtained in species reproducing by the normal sexual mode. In this case, the main cytogenetic features of gynogenesis, inactivation of male chromosomes and prevention of female chromosome set reduction, are achieved by special experimental treatments. Inactivation of male chromosomes is induced by irradiation of sperm. X-rays, gamma rays, or ultraviolet (UV) irradiation may be used for this purpose. This method of chromosome inactivation is based on different sensibilities of chromosomes and cytoplasmatic structures of spermatozoa. At high dosage of irradiation, chromosomes are inactivated but spermatozoa maintain ability for movement and insemination of eggs.

After insemination of eggs with genetically inactivated spermatozoa, gynogenetic haploids are produced. Haploids in fish are morphologically abnormal ('haploid syndrome') and die before or soon after hatching. In order to obtain viable diploid gynogenetic fish, the haploid female chromosome set must be doubled. A female chromosome set may be doubled in two ways: either by suppression of the second meiotic division in eggs (meiotic gynogenesis) or by suppression of the first mitotic division in haploid embryos (mitotic gynogenesis). For artificial suppression of meiotic or mitotic divisions, strong physical treatments (shocks) are applied to the embryos. Most commonly used treatments are either low or high temperatures (cold and heat shocks) or hydrostatic pressure. For suppression, shock is usually applied at anaphase of the corresponding division. Under the influence of this treatment, spindle fibers are destroyed and, as a result, division stops and daughter products are fused. When the second meiotic division is suppressed, the second polar body is not extruded; it is fused with the haploid female pronucleus. During suppression of the first mitotic division in haploid embryos, two haploid nuclei are united to form a diploid nucleus. After the next mitotic cycle two diploid blastomeres are formed. With low frequency (usually less than 0.1%) viable gynogenetic diploids may appear without shock application due to sporadic spontaneous suppression of the second meiotic division in eggs (meiotic gynogenesis).

The first data on meiotic gynogenesis in common carp were reported by the Russian scientists Romashov et al. (1960) and Golovinskaya et al. (1963). These scientists revealed that after insemination of eggs by sperm irradiated with high dosages of X-rays (100–200 kR) spontaneous viable gynogenetic diploids appeared with low frequency. Golovinskaya and Romashov (1966) investigated segregation among gynogenetic fish with regard to scale cover types. On this basis it was correctly suggested that: (1) gynogenetic diploids appeared due to suppression of the second meiotic division in eggs and (2) heterozygosity in gynogenetic fish resulted from crossing over in the chromosome region between gene and centromere during first meiotic division in oocytes.

Investigations of meiotic gynogenesis in common carp were continued in studies by Cherfas (1975, 1977), Cherfas and Truveller (1978), Nagy et al. (1978, 1979, 1983), Nagy and Csanyi (1978, 1982), Gomelsky et al. (1979, 1989, 1992, 1996), Taniguchi et al. (1986), Hollebecq et al. (1986), Linhart et al. (1986, 1987), Komen et al. (1988), Cherfas et al. (1990, 1993a, 1994a,b), Sumantadinata et al. (1990), Kim et al. (1993), Shelton and Rothbard (1993), Khan et al. (2000) and others. The main data on meiotic gynogenesis in common carp are summarized below.

(a) Techniques for production of meiotic gynogenetic progenies in common carp have been optimized. In initial studies X-rays or gamma rays were used for

genetic inactivation of sperm. Later, UV-irradiation was used more frequently for this purpose mainly due to its simplicity. Suppression of the second meiotic division in eggs was achieved by application of either cold or heat shocks. To standardize the timing of shock application for different pre-shock temperatures, the pre-shock time was frequently expressed in terms of mitotic interval τ_0 (proposed by Dettlaff and Dettlaff, 1961). Optimal timing for suppression of the second meiotic division was about 0.05–0.15 τ_0 and 0.10–0.20 τ_0 after insemination for cold and heat shock, respectively.

(b) Meiotic gynogenetic progenies were all-female. This proved female homogamety (females—XX, males—XY) in common carp. Induced gynogenesis is not used for direct production of all-female progenies due to possible inbreeding depression and relative complexity. However, all-female meiotic gynogenetic progenies were frequently used as initial material in experiments on hormonal sex reversal (see below).

(c) As mentioned above, in the case of meiotic gynogenesis, heterozygosity results from crossing over between gene and centromere. Frequency of crossing over depends on the distance between a given gene and centromere and may differ to a great extent. Frequency of heterozygotes in meiotic gynogenetic progenies in common carp varies from 0.05 to 0.99 for different genes. It is estimated that average heterozygosity per locus is about 0.4. On this basis, coefficient of inbreeding (F) for the first meiotic gynogenetic generation in common carp is about 0.6, higher than the value of F for self-fertilization (0.5). Therefore, it was suggested using meiotic gynogenesis for development of inbred lines for future application in practical breeding (Golovinskaya 1968; Nagy and Csanyi 1978). The rate of homozygosity increase diminishes in successive generations of meiotic gynogenesis, while genetic identity increases more rapidly. Tissue transplantation showed that the fourth successive gynogenetic generation was genetically identical (Nagy et al., 1983).

The technique for production of diploid gynogens in common carp by suppression of the first mitotic division in haploid embryos (mitotic gynogenesis) was elaborated later than development of meiotic gynogenesis. Mitotic gynogenesis in common carp has been reported by Nagy (1987), Linhart et al. (1987), Gomelsky et al. (1989, 1992, 1998), Komen et al. (1991), Rothbard (1991), Sumantadinata et al. (1990), Cherfas et al. (1993a,b, 1994b), Yousefian et al. (1996) and others.

The main data on mitotic gynogenesis in common carp are summarized below.

(a) Suppression of the first mitotic division in haploid embryos was usually achieved by application of heat shock. Optimal timing for heat shock application was about 1.5–1.9 τ_0 after insemination.

(b) As a rule, mitotic gynogenetic progenies were all-female. However, a recessive mutation of sex-determining gene has been identified (Komen et al., 1992), which resulted in the appearance of males in some gynogenetic progenies.

(c) Diploids obtained by mitotic gynogenesis are homozygous for all genes ($F = 1.0$). This method provides an opportunity for obtaining clones.

For the first time, Komen et al. (1991) achieved production of two types (homozygous and heterozygous) of clones in common carp. The genetic identity of fish clones was proved by tissue transplantation test. Later, clones in common carp were also obtained, as reported, by Ben-Dom et al. (2001). In this study DNA fingerprinting and mixed leukocyte reaction were used for the confirmation of genetic identity of cloned fish.

3. Induced androgenesis

Androgenesis is embryo development under the control of only paternal chromosomes. Genetic inactivation of female chromosomes for induction of androgenetic development in fish may be achieved by irradiation of eggs. After insemination of eggs with genetically inactivated chromosomes by intact spermatozoa, androgenetic haploids are produced. To produce viable diploid androgenetic fish, the paternal haploid chromosome set must be doubled. This may be achieved by suppression of the first mitotic division in the haploid embryos.

Diploid androgenesis in common carp has been reported by Grunina et al. (1990). Female chromosomes were inactivated by irradiation of eggs with X-rays; diploidy was restored by suppression of the first mitotic division in androgenetic haploids with heat shock. Bongers et al. (1994) succeeded to induce androgenetic development in common carp by UV-irradiation of eggs stirred in a synthetic ovarian fluid; the diploidy was induced by heat shock. In further studies (Grunina et al., 1995; Bongers et al., 1999) viable androgenetic YY males were identified, that after crossing with normal females (XX), produced all-male progenies. Androgenesis has been used for obtaining of clones in common carp (Bongers et al., 1995).

Bercsenyi et al. (1998) successfully performed interspecies androgenesis between common carp and goldfish by insemination of genetically inactivated common carp eggs with goldfish sperm and subsequent doubling of goldfish haploid chromosome set.

4. Induced polyploidy

Induced polyploidy is artificial production of individuals, with an increased number of haploid chromosome sets. In aquaculture and fisheries, this method is primarily used for production of triploid fish, i.e. fish whose karyotypes contain three haploid chromosome sets. Triploid fish are genetically

sterile, i.e. they are not capable of producing viable progeny. Also triploids are characterized by complete or partial reduction of gonads. Abnormalities in development of reproductive systems in triploids are caused by the presence of a third, 'additional' haploid chromosome set, which disturbs the normal process of conjugation and disjunction of homologous chromosomes.

There are several methods for producing triploid fish. The simplest and better developed method is based on suppression of the second meiotic division in eggs after insemination by intact spermatozoa. Production of triploids by this method has been elaborated along with the technique for inducing meiotic gynogenesis, since both methods use similar shocks for doubling of the female chromosome set. Shock application should provide a high frequency of triploids, but at the same time does not decrease drastically embryo survival. Cold shocks were used frequently in initial studies on induced triploidy in common carp (Ojima and Makino, 1978; Gervai et al., 1980; Ueno, 1984; Taniguchi et al., 1986; Cherfas et al., 1990; Linhart et al. 1991); in further investigations (Hollebecq et al., 1988; Recoubratsky et al., 1989, 1992; Gomelsky et al., 1992; Cherfas et al., 1993a, 1994c; Basavaraju et al., 2002) heat shocks were mainly applied. From a practical point of view, application of heat shocks appears to be more convenient for mass production of triploid progenies since duration of heat shock is much shorter than duration of cold shock. Recoubratsky et al. (1989, 1992) described the method for mass production of triploid common carp using heat shock. The following parameters were recommended as optimal: timing—0.2 τ_0 after insemination (about 6 min at pre-shock temperature 20 °C), shock temperature and duration—40 °C and 2 min, or 41 °C and 1.5 min. These treatments provided 80–100% triploids in progenies with embryo survival 50–70% of controls.

As already mentioned, triploid fish are characterized by a reduction in gonad development. This makes triploids potentially valuable for commercial rearing. It was speculated that, during the rearing of triploids, there would not be retardation in somatic growth, usually recorded in diploid fish during the period of gonad maturation. Theoretically, this advantage should be especially profound when sex maturation is reached before fish reach a marketable size. Cherfas et al. (1994c) and Basavaraju et al. (2002) assessed triploid common carp for culture in such conditions. A reduction in gonad development (especially ovaries) in triploid fish was observed and verified in both studies. However, the reduction in gonad development in triploids did not result in an increase of somatic growth rate. Moreover, triploid fish grew slower than diploid fish in almost all comparative trials (Cherfas et al., 1994c; Basavaraju et al., 2002). These data cast doubt on the potential of triploid common carp for commercial rearing.

The second method of production of triploid fish is the crossing of previously obtained tetraploid fish with normal diploid fish. Tetraploid fish can be obtained by suppression of the first mitotic division in normal diploid embryos. This method of triploid fish production was realized in rainbow trout (Chourrout et al., 1986). Recoubratsky et al. (1989) and Cherfas et al. (1993a) have reported results of successful experiments on mass production of tetraploid common carp larvae by application of heat shocks; in some trials the frequency of tetraploids in progenies was up to 100%. However, attempts to raise common carp tetraploids have demonstrated their very poor survival. Only three tetraploid fingerlings have been reported (Cherfas et al., 1993a).

5. Sex control

As mentioned previously, artificial sex regulation in fish is connected mainly with elaboration and application of the hormonal sex reversal method. Hormonal sex reversal (or inversion) is a change from the normal process of sex differentiation under the influence of steroid sex hormones so that genotypic females develop testes or genotypic males develop ovaries. Sex reversal changes only fish phenotype, the genotypic formula of sex chromosomes remains the same. Hormonal sex reversal may be used in two different ways for sex regulation. The direct method for producing monosex populations involves hormonal treatment of all reared fish during the period of sex differentiation. The indirect method, or genetic sex regulation, involves crossing normal fish with previously obtained sex-reversed fish. In the case of female homogamety, which has been revealed in common carp, all-female progenies may be produced by crossing normal females XX with sex-reversed XX males (neomales) obtained by phenotypic hormonal sex reversal of genotypic females. Genetic sex regulation is regarded as the preferred method since there is no need to treat all reared fish with a hormone and hormonally treated fish are not intended for human consumption.

Nagy et al. (1981) first reported successful sex reversal of genotypic females of common carp by androgen treatment. The androgen, methyltestosterone (MT), was added to a prepared diet at a dose of 100 mg kg^{-1} and orally administrated to all-female gynogenetic carp at different 36-day periods. It was shown that androgen treatment during any 36-day period beginning from 8 to 62 days after hatching resulted in 70–90% males in experimental groups while the control group of fish consisted of only females. Mature sex-reversed males were crossed with normal females and produced all-female progenies.

Gomelsky (1985) used the same dosage of MT (100 mg kg^{-1} of diet) to induce hormonal sex reversal in two consecutive generations of common carp: all-female gynogenetic progeny and all-female progeny obtained by crossing of gynogenetic sex-reversed males with normal females. Initiation of a 40-day MT treatment for these two groups was 60 and 76 days after hatching, respectively; the resulting percentage of males was 85% and 51%. Histological study of sex reversal process in genotypic females revealed that androgen treatment caused cytological differentiation of sex cells in the male direction; along with spermatogenesis, mor-

phological transformation of primary ovary to normal testes has occurred. It was shown also (Gomelsky, 1985) that reversed males did not differ from normal males of common carp with regard to the volume of milt and fertilizing ability of spermatozoa.

Komen et al. (1989) have studied the effects of 5-week oral administration of MT (doses of 50 and 100 mg kg^{-1}) on sex ratio in normal mixed-sex progeny of common carp; initiation of androgen treatment was 3, 6, or 10 weeks after hatching. Best results (92.7% of males in experimental group as compared to 64.4% in control) were obtained after administration of 50 mg kg^{-1} MT in the food between 6 and 11 weeks after hatching; earlier or later treatments with MT at concentrations 50 and 100 mg kg^{-1} resulted in high percentages of sterile fish. It was noted (Komen et al., 1989) that for a more precise description of the hormonal treatment procedure the amount of MT per unit of body weight or per unit of body weight gain should be determined.

Shelton (1990) and Shelton et al. (1995) proposed a conceptual model for common carp that considers both age and size in relation to gonad differentiation. According to this model, sex reversal effectiveness assumes a relationship between delivery of a pharmacologically effective dose (PED) over a period of gonadal differentiation that is influenced by age as well as size of fish. Growth trajectory is controlled by the environmental factors of stocking density, temperature and food availability.

Gomelsky et al. (1994), on the basis of experimental results on hormonal sex reversal in common carp obtained under different climatic conditions, suggested that weight of the fish (rather than their age) may be used as a practical criterion for determining the appropriate period of androgen treatment. In the most successful experiments, the initial (at the beginning of androgen treatment) fish weight varied from 2.7 to 9.2 g while initial fish age varied from 27 to 65 days after hatching. Also it was shown that effectiveness of androgen treatment depended on the conditions of fish rearing; results of experiments have usually been better when experimental fish were kept in recirculation water systems.

As compared to other methods, genetic production of all-female progenies in common carp by crossing of sex-reversed and normal fish shows the most promise for practical aquaculture. Sex-reversed males of common carp do not show abnormalities such as those observed in development of sperm ducts in rainbow trout (Bye and Lincoln, 1986; Geffen and Evans, 2000). This permits the stripping of sperm from sex-reversed males and its use for mass production of all-female progenies. Cherfas et al. (1996) reported results of comparative rearing of all-female and normal mixed-sex progenies in situation where fish reach sexual maturity before reaching market size. It was shown that rearing of all-female progenies increased production yield by 7–8%. This increase was attributed to sexual dimorphism in body weight: females were 15% heavier than males. An additional advantage of rearing all-female progenies is prevention of uncontrolled fish reproduction.

6. Distant hybridization

There are a few cases where distant hybridization results in polyploidy of fish without application of any physical treatments. Vasilyev et al. (1975) showed that triploids are rare survivors from crosses of common carp females with males of grass carp (*Ctenopharyngodon idella*); they appear to be due to sporadic spontaneous suppression of the second meiotic division in eggs. Triploidy in hybrids between koi females and rosy barb (*Barbus conchonius*) males was also reported by Varadi et al. (1995).

Triploidy of fish may also result from backcross hybridization. Ojima et al. (1975) showed that about half of the backcross hybrids obtained from crossing F_1 hybrid females (crucian carp, *Carassius auratus cuvieri* × common carp) with males of common carp were triploids.

Cherfas et al. (1994d) summarized results of long-term studies on induced gynogenesis and polyploidy in hybrids between silver crucian carp (*Carassius auratus gibelio*) and common carp. The ability of F_1 hybrid females to produce diploid (i.e. with unreduced chromosome number) eggs was responsible for the high yield of spontaneous (without application of any shocks) diploids in gynogenetic progenies obtained from F_1 females and for triploidy in hybrids from backcrosses of F_1 females with males of parental species (Cherfas and Ilyasova, 1980; Cherfas et al., 1981). Diploid hybrid females have been reproduced by induced gynogenesis during several consecutive generations (Cherfas et al., 1994d). Cytogenetic studies (Emelyanova and Cherfas, 1980; Emelyanova, 1984) have shown that the ability of diploid hybrid females to produce eggs with unreduced chromosome number was caused by additional chromosome endoreduplication in early oogenesis. Gomelsky et al. (1985, 1988) demonstrated that in contrast to the sterile F_1 hybrid males (XY) reversed males (XX), obtained by hormonal sex reversion of genotypic hybrid females, were fertile and produced unreduced diploid spermatozoa. By crossing of diploid hybrid females (XX) and reversed males (XX) all-female (XXXX) allotetraploid (amphidiploid) hybrids were obtained. Tetraploid hybrids also produced diploid gametes; by crossing tetraploid females with tetraploid reversed males, a second generation of tetraploid hybrids has been obtained.

Recently Liu et al. (2001) reported formation of tetraploid stocks of red crucian carp (*Carassius auratus* red var.) and common carp hybrids. In this study, tetraploidy also occurred from the ability of diploid hybrids to produce unreduced diploid gametes. The authors managed to produce a bisexual tetraploid population, which was reproduced inter se during several generations. Apparently, this is the first case of artificial synthesis of the bisexual hybrid tetraploid form in fish.

7. Conclusion

Techniques for production of gynogenetic, androgenetic, triploid, and monosex progenies in common carp have been

elaborated. Female homogamety in this species was revealed on the basis of all-femaleness of gynogenetic progenies. Induced gynogenesis results in the increase of homozygosity; the rate of this increase depends on the type of gynogenesis. Mitotic gynogenesis was used for production of clones in common carp. In androgenetic progenies of common carp, YY males have been identified, that after crossing with normal females (XX) produced all-male progenies. Triploids of common carp are characterized by a significant reduction in gonad development (especially ovaries). However, reduction in gonad development did not result in an increase of somatic growth rate in these fish. The procedure of androgen treatment for inducing phenotypic sex reversal in genotypic females (XX) has been elaborated. All-female progenies of common carp were produced on a large scale by crossing normal females (XX) with hormonally sex-reversed males (XX). Rearing of all-female progenies in conditions when fish normally reach sexual maturity before reaching of market size increased production yield by 7–8%. As compared to other methods, genetic production of all-female progenies in common carp by crossing of sex-reversed and normal fish shows the most promise for practical aquaculture. In a few cases distant hybridization resulted in polyploidy of fish without application of any physical treatment. Ability of hybrid females between crucian carp and common carp to produce diploid (i.e. with unreduced chromosome number) gametes provided an opportunity to produce triploid and tetraploid hybrid progenies.

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